M_1 Muscarinic Acetylcholine Receptor Agonism Alters Sleep without Affecting Memory Consolidation

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Abstract

■ Preclinical studies have implicated cholinergic neurotransmission, specifically M_1 muscarinic acetylcholine receptor (mAChR) activation, in sleep-associated memory consolidation. In the present study, we investigated the effects of administering the direct M_1 mAChR agonist RS-86 on pre–post sleep memory consolidation. Twenty healthy human participants were tested in a declarative word-list task and a procedural mirror-tracing task. RS-86 significantly reduced rapid eye movement (REM) sleep latency and slow wave sleep (SWS) duration in comparison with placebo. Presleep acquisition and postsleep recall rates were within the expected ranges. However, recall rates in both tasks were almost identical for the RS-86 and placebo conditions. These results indicate that selective M_1 mAChR activation in healthy humans has no clinically relevant effect on pre–post sleep consolidation of declarative or procedural memories at a dose that reduces REM sleep latency and SWS duration. ■

INTRODUCTION

A strong line of research indicates that cholinergic neurotransmission is a critical component for processes mediating long-term plasticity and memory consolidation (Power, Vazdarjanova, & McGaugh, 2003). A global decrease in cholinergic tone, either as part of a pathologic decline, such as in Alzheimer's disease (Coyle, Price, & DeLong, 1983), or experimentally induced by anticholinergic compounds, such as scopolamine (Sitaram et al., 1978), consistently disrupts memory function. In turn, improved memory can be found after increasing acetylcholine (ACh) levels with ACh esterase (AChE) inhibitors, such as physostigmine (Davis et al., 1978) or donepezil (Courtney et al., 2004). The potential for developing treatments for memory deficits, such as those in Alzheimer's disease, that target cholinergic neurotransmission has enhanced interest in understanding cholinergic involvement in cognitive functioning (Fisher et al., 2003; Tune & Sunderland, 1998; Coyle et al., 1983).

Slow wave sleep (SWS) and rapid eye movement (REM) sleep are characterized by differing brain ACh levels (Mendelson, 2001) and may have dissociable roles in memory consolidation (Walker & Stickgold, 2004; Plihal & Born, 1997). Experimental work elucidating the involvement of cholinergic neurotransmission in sleep-associated memory consolidation, however, is just beginning to emerge (Hasselmo, 1999).

There is substantial experimental support for the view that SWS and REM sleep may strengthen distinct types of memories (Walker & Stickgold, 2004; Plihal & Born, 1997). Sleep periods rich in SWS enhanced predominantly declarative memories, for instance, word pairs (Plihal & Born, 1997; Fowler, Sullivan, & Ekstrand, 1973). Preclinical studies provided evidence that declarative memory traces temporarily stored in the hippocampus (Leutgeb et al., 2005) are reactivated during SWS and are ultimately transferred to the neocortex for long-term storage (Sutherland & McNaughton, 2000). This dialogue is thought to require a down-regulation of ACh release during SWS (Buzsaki, 1986) so that suppression of feedback signals from excitatory hippocampal efferents may be removed (Hasselmo, 1999; Hasselmo, Schnell, & Barkai, 1995; Buszaki, 1989). Consistent with this view, Gais and Born (2004) recently found in humans that disrupting SWS-associated ACh down-regulation with the AChE inhibitor physostigmine disrupted declarative memory consolidation of a word-pair task.

Unlike SWS, REM sleep is characterized by a high ACh tone (Hobson & Pace-Schott, 2002; Lydic & Baghdoyan, 1993). REM sleep appears to strengthen nondeclarative memory, such as that mediating procedural mirror-tracing (Fischer, Hallschmid, Elsner, & Born, 2002; Plihal & Born, 1997). However, there is some evidence that REM sleep involvement may be related to memory

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complexity or task difficulty rather than memory type per se (Smith, 1996). Elucidating the involvement of cholinergic neurotransmission, specifically AChR subtype functioning, in sleep-associated memory consolidation would be informative for the understanding of sleep-related memory processes and may enable targeted cognitive enhancing therapeutics to be developed (Tune & Sunderland, 1998).

The present study examined the involvement of M_1 muscarinic AChRs (mAChRs) in pre–post sleep memory consolidation because this subtype is the major excitatory postsynaptic mAChR in the brain and is of particular interest for memory processes (Anagnostaras et al., 2003; Ferreira et al., 2003; Power, McIntyre, Litmanovich, & McGaugh, 2003; Cortes & Palacios, 1986; Cortes, Probst, Tobler, & Palacios, 1986). The direct M_1 mAChR agonist RS-86 was administered posttraining to healthy human participants subjected to both declarative and procedural memory tasks in the evening prior to sleep. We hypothesized that the elevated M_1 mAChR activity would suppress SWS and enhance REM sleep (Riemann et al., 1988; Spiegel, 1984), and that this would disrupt the consolidation of declarative memories and improve procedural learning.

METHODS

Subjects

Twenty healthy volunteers, 10 men and 10 women, aged 21 to 37 years (mean ± *SEM*, 27.1 ± 1.0 years) participated in the present study. All participants underwent an extensive physical examination, including an electrocardiogram, electroencephalogram, and routine laboratory screening to rule out any somatic disorders. In addition, a urine drug screening demonstrated that all participants were free of any benzodiazepines, barbiturates, amphetamines, or opiates. Only women not pregnant or at risk of becoming pregnant were included. No participant presented any contraindication for cholinergic compounds. All subjects underwent an extensive psychiatric evaluation, including a Structured Clinical Interview for Diagnosis and a thorough clinical evaluation of the family history by an experienced psychiatrist. Subjects with a personal or family history of psychiatric disorders or primary sleep disorders according to *DSM-IV* were excluded. All participants were completely free of any medication, drank no alcohol during the period of the study, and were nonsmokers. Caffeine intake was limited to one cup of coffee in the morning after the recall test. A sleep diary ensured that subjects' typical sleep schedules approximated the imposed sleep schedule in the laboratory. All participants were informed in detail and provided written informed consent prior to the study. The study was carried out in accordance with the Declaration of Helsinki and was approved by the local ethic committee.

Experimental Design

The present analysis was associated with a study on the effects of cholinomimetics on REM sleep regulation (Nissen et al., 2006). All participants spent three nights in the sleep laboratory. The first night served as adaptation to the laboratory conditions and was used to rule out any sleep abnormalities. In the second and third nights, participants received 1.5 mg RS-86 or placebo at 10:00 p.m., 1 hr prior to ''lights-off'' time at 11:00 p.m. The administration of RS-86 and placebo followed a randomized double-blind protocol. Two memory tasks were performed at 9:30 p.m. prior to sleep (learning condition) and on the following morning between 7:30 and 8:00 a.m. (recall condition), that is, 30 to 60 min after awakening and ''lights-on'' time at 7:00 a.m.

RS-86

The spiro-piperidyl derivate RS-86 is a direct M_1 mAChR agonist with low or no affinities to other receptors (Rupniak, Tye, & Iversen, 1992; Wanibuchi et al., 1990; Palacios et al. 1986). RS-86 is efficiently absorbed with oral administration, reaches peak plasma concentrations in 2 to 3 hr, and has an elimination half-life of 6 to 8 hr (Spiegel, 1984). Due to its pharmacodynamic profile, RS-86 causes no or only minor peripheral side effects in humans in doses up to 2 mg. Side effects in higher doses include cholinomimetic symptoms, such as increased salivation, sweating, nausea, or diarrhea. No serious side effects were observed in human studies using up to 20 mg (Spiegel, 1984). Based on pharmacological data and following previous studies (e.g., Riemann et al., 1988), 1.5 mg RS-86 was administered orally at 10:00 p.m., 1 hr prior to ''lights-off'' time at 11:00 p.m. RS-86 is known to reduce REM latency and attenuate SWS without disturbing sleep continuity (Riemann et al., 1988; Spiegel, 1984).

Memory Tasks

A word-list task was used to assess declarative memory consolidation. A mirror-tracing task was used to investigate procedural learning.

Word-list

Two versions of a 15-item word-list were taken from the German version of the Rey Auditory–Verbal Learning Test (RAVLT; Rey, 1941, 1964) to enable repeated measurements in the two experimental nights. In the evening, the test list was read aloud to the participants in five trials according to the procedures outlined by Lezak (1982). Each trial involved reading of the test list followed by free recall of the words in any order. For each trial, the number of correctly retrieved words was noted.

The following morning, participants were given a single test trial in which they were asked to recall as many words as possible from the same list without reading the list again. In contrast to the standard RAVLT procedures, no interference list and no recognition condition were used. As outcome measures for acquisition in the evening learning condition, the percentage of correctly retrieved items in the first and fifth evening trials was calculated. As a measure of presleep versus postsleep recall rate, the percentage of correctly retrieved words in the morning trial relative to the fifth evening trial was calculated.

Mirror-tracing Task

The mirror-tracing procedures in the present study, including the line-drawn stimuli, followed precisely the procedures outlined by Plihal and Born (1997). Participants were asked to trace different line-drawn stimuli using a stylus with an electronic light sensor that measures (1) draw time, (2) number of errors, and (3) total error time. Errors refer to events when the stylus left the trace. Visual access to the stimuli was provided only indirectly via a mirror. During the learning condition in the evening, mirror-tracing a star was repeated until the participant reached a criterion of a maximum of six errors. Then, six line-drawn figures (Plihal & Born, 1997) were presented one after the other. During the recall condition in the following morning, subjects were asked to mirror trace the star one single time to keep conditions comparable. Then, the six figures of the preceding evening were presented. Different stimuli sets were used in the two experimental nights. Learning performance was assessed by measuring (1) the number of trials to criterion for tracing the star, (2) the mean draw time, (3) the mean error time, and (4) the mean total error counts. The three latter measures were evaluated separately for mirror-tracing the star and the six figures. During the recall period in the following morning, the same measures were assessed. Sleep-associated improvement was defined as the individual difference for the measures of (1) mean draw time, (2) mean error time, and (3) mean error count between the evening/learning and the morning/recall sessions. Finally, this improvement was expressed as a percentage with the respective values obtained during learning set to 100%.

Sleep Recordings

Sleep recordings were performed from 11:00 p.m. to 7:00 a.m. and scored according to standard criteria (Rechtschaffen & Kales, 1968) by experienced and experimentally blind raters. All raters participated in weekly meetings to discuss and resolve any scoring issues. Interrater reliability for all facility technicians involved in scoring polysomnograms is checked bimonthly. The agreement coefficient between any two raters is required to be >85%. The following variables of *sleep continuity and architecture* were assessed: sleep onset latency was defined as the period between when the lights were turned out and the first 30-sec epoch stage 2 sleep; sleep period time (SPT) was defined as the period between sleep onset and the final awakening; sleep efficiency (SE) was defined as the ratio of time in bed to total sleep time × 100%; time spent in waking referred to the percent of SPT (%SPT) spent in the waking state; time spent in sleep stages 1, 2, SWS (stages 3 and 4), and REM sleep referred to the percent of SPT spent in each. In addition, SWS (%SPT) was calculated separately for the first (11 p.m.–3 a.m.) and second (3 a.m.–7 a.m.) half of the night. REM latency was defined as the period between sleep onset and the occurrence of the first 30-sec epoch of REM sleep, including intermittent waking times. The number of rapid eye movements (NREMs) indicated the number of REMs across the whole night.

Data Analysis

The mean values of the data are presented with the standard errors of the mean (*SEM*s). Multivariate analyses of variance (MANOVAs) were conducted, with the two treatment conditions (placebo, RS-86) as repeated measures. Follow-up post hoc contrasts were calculated to determine the source of significance detected in the MANOVA. To test correlational hypotheses, Pearson's correlation coefficients were calculated. All statistical analyses were performed with the SPSS 12-program package. The level of accepted significance was $p < .05$ (two-tailed).

RESULTS

Sleep

Results from the sleep recordings are summarized in Table 1. A multivariate test (Wilks' Lambda) revealed no substance effects on the listed parameters, $F(8,12) = 1.8$, $p = .180$. In accordance with previous analyses that demonstrated a significant reduction of REM latency and SWS after administration of RS-86 (Riemann et al., 1988; Spiegel, 1984), we performed univariate tests (Greenhouse–Geisser). These demonstrated that RS-86 reduced cumulative SWS duration (%SPT) and REM latency relative to placebo (Figure 1A and B). SWS (%SPT) in the first half of the night (11 p.m.–3 a.m.) was significantly attenuated after administration of RS-86. SWS (%SPT) in the second half of the night (3 a.m.–7 a.m.) was not significantly altered ($p = .074$). Other sleep parameters were not significantly influenced. Specifically, sleep continuity did not differ between the RS-86 and placebo conditions.

Table 1. Effects of RS-86 on Sleep Continuity and Architecture

	Substance		*MANOVA*	
Measure	*Placebo*	*RS-86*	*F*	*p*
Sleep latency	21.3 ± 4.7	15.0 ± 1.8	1.5	.230
SPT	453.6 ± 6.0	462.8 ± 2.8	2.1	.167
Sleep efficiency	89.4 ± 1.4	91.2 ± 0.9	1.1	.303
Waking (%SPT)	5.6 ± 0.7	5.5 ± 0.7	0.0	.879
Stage 2 (%SPT)	54.0 ± 1.4	55.7 ± 1.6	2.8	.112
SWS (%SPT)	10.1 ± 1.7	7.7 ± 1.7	9.1	**.007**
SWS (%SPT), 11 p.m.–3 a.m.	17.9 ± 2.8	14.3 ± 3.1	8.1	**.010**
SWS (%SPT), 3 a.m.–7 a.m.	2.8 ± 0.8	1.4 ± 0.5	3.6	.074
REM (%SPT)	21.7 ± 1.0	22.8 ± 1.1	1.7	.213
NREMs	494.4 ± 31.2	513.2 ± 39.4	0.5	.480
REM latency	85.3 ± 11.0	62.4 ± 9.4	6.0	**.024**

SPT = sleep period time; NREMs = number of rapid eye movements.

Univariate tests (Greenhouse–Geisser); significant comparisons are in **bold**.

Table 2. Mirror-tracing Performance

		Substance			
Session	*Measure*	*Placebo*	*RS-86*	*F*	*p*
Learning	Star				
	Trials to criterion	2.3 ± 0.7	3.1 ± 0.7	0.57	.461
	Draw time	48.2 ± 9.0	51.2 ± 9.2	0.05	.827
	Error time	4.7 ± 1.6	4.6 ± 1.3	0.00	.981
	Errors	4.8 ± 1.5	9.0 ± 2.6	1.55	.228
	Six figures				
	Draw time	65.5 ± 5.5	66.4 ± 6.0	0.02	.900
	Error time	5.9 ± 1.1	4.7 ± 1.1	0.97	.336
	Error	7.3 ± 1.4	7.4 ± 1.6	0.01	.947
Recall	Six figures				
	Draw time	50.6 ± 3.8	52.1 ± 3.3	0.41	.531
	Error time	2.2 ± 0.5	2.6 ± 0.6	0.91	.352
	Errors	3.6 ± 0.8	3.9 ± 0.8	0.37	.551
	Improvement				
	Draw time	14.9 ± 2.9	14.3 ± 3.8	0.01	.916
	Error time	3.7 ± 0.9	2.1 ± 0.7	1.76	.200
	Errors	3.7 ± 1.3	3.6 ± 1.1	0.00	.949

Times are given in seconds. Trials and errors are given in numbers. Improvement is relative to learning session.

Procedural Memory Consolidation

Table 2 summarizes the subjects' mean performance data on the mirror-tracing tasks during the learning and recall sessions. None of the performance measures (draw time, error time, or number of errors) during the *learning period* prior to sleep differed between the placebo and RS-86 conditions. No differences in the absolute or relative performance measures during the *recall period* in the morning were evident between the placebo and RS-86 conditions. Likewise, analysis of the percent improvement from presleep to postsleep performance did not reveal a treatment effect (Figure 2A). In the placebo condition, draw time decreased by 22.7 ± 2.8%, error time by 62.7 ± 14.7%, and the number of errors by 50.7 ± 13.3%. Similarly, in the RS-86 condition, draw time decreased by 21.5 ± 3.5%, error time by 44.7 ± 13.5%, and the number of errors by 48.6 ± 12.4% ($F = 0.31$, $p = .585$; $F = 1.10$, $p = .307$; and $F = 0.49$, $p = .494$ vs. respective placebo values). Thus, subjects showed similar improvement in the

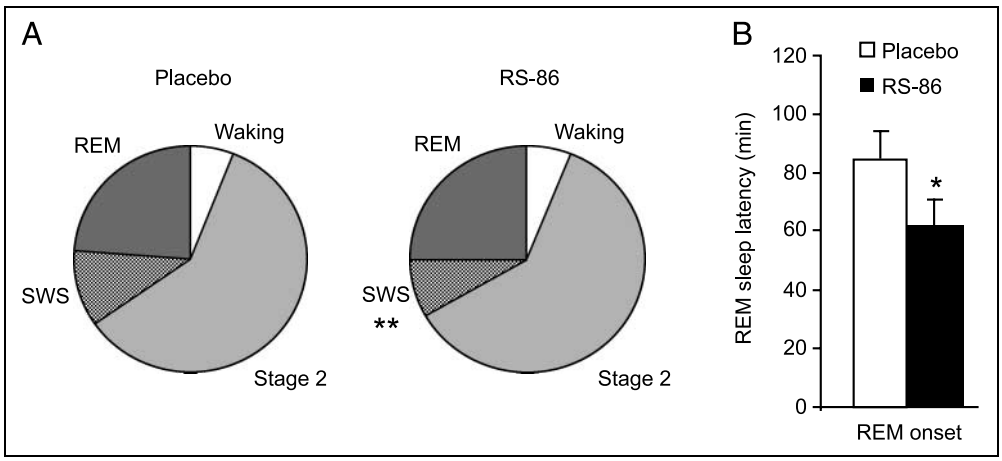

Figure 1. Administration of RS-86 alters sleep architecture. (A) Decreased SWS duration (**$p < .01$), shown as percent sleep period time (%SPT), and (B) decreased REM sleep latency (*$p < .05$) were observed in the RS-86 relative to the placebo condition. Data are shown as mean REM latencies ± *SEM*s.

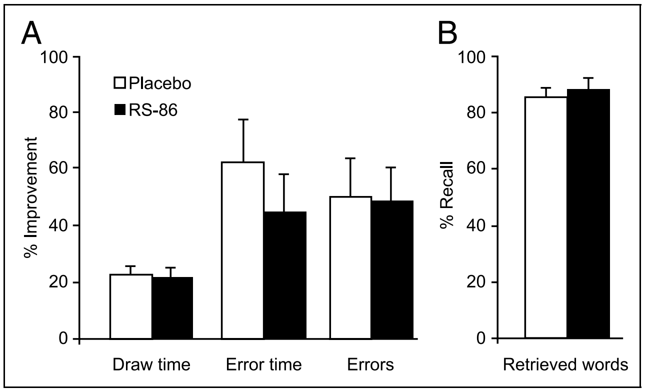

Figure 2. Administration of RS-86 did not affect memory consolidation. (A) In the mirror-tracing procedural memory task, no treatment effects were observed on sleep-associated improvement of draw time, error time, or number of errors (ps > .05). Data are shown as percent change from the presleep learning trial to the postsleep recall trial. (B) In the RAVLT declarative memory task, no treatment effect was observed on the percent of words recalled (p > .05). The number of words correctly recalled during the final (fifth) learning trial was set as 100%, and postsleep recall performance is shown as the percent of words retrieved relative to the final learning trial. All data are shown as mean values ± *SEM*s.

performance of the mirror-tracing task according to all measures.

Declarative Memory Consolidation

Table 3 summarizes the subjects' mean performance data on the RAVLT word-list task during the learning and recall sessions. Performance during the *learning period* prior to sleep did not differ between the placebo and RS-86 conditions. Similar to the negative findings in the procedural memory task, there was no difference in the number of correctly retrieved words during the *recall period* in the morning between the placebo and RS-86 conditions. Our analysis of the percent of recalled words after sleep, relative to the number of words recalled before sleep (i.e., number words recalled during the fifth learning trial set as 100%), did not reveal a substance effect (Figure 2B). The percent of correctly retrieved words relative to the presleep performance baseline was 85.5 ± 3.1% in the placebo condition and 88.6 ± 3.0% in the RS-86 condition (F = 0.84, p = .372). Hence, measures of absolute and relative recall rates were almost identical after administration of placebo or RS-86 in this word-list declarative memory task.

Relationship between Sleep and Memory Data

The RS-86 induced reduction in REM latency and decline in SWS sleep (expressed in %) did not correlate with pre–post sleep changes in performance of either the declarative or the procedural memory task (expressed in %; Pearson's correlation coefficient; level of significance p > .2; data not shown).

Table 3. Auditory Verbal Learning Task (AVLT) Performance

Response Measure	*Placebo*	*RS-86*	*F*	*p*
Learning				
Correctly retrieved words, T5	13.9 ± 0.3	13.9 ± 0.2	0.04	.841
Recall				
Correctly retrieved words	12.0 ± 0.6	12.4 ± 0.5	0.59	.451

T5 = fifth trial.

DISCUSSION

The present study examined the involvement of M_1 mAChRs in pre–post sleep performance on a declarative and procedural memory task in humans. The results did not support our initial hypothesis that the consolidation of declarative and procedural memories would be affected by M_1 mAChR activation. Similar to previous studies (Riemann et al., 1988; Spiegel, 1984), administration of the direct M_1 mAChR agonist RS-86 reduced REM sleep latency and SWS duration relative to placebo (Table 1). Acquisition performance in the evening and recall performance in the morning in both the declarative word-list task and the procedural mirror-tracing task fell within the expected ranges (Phihal & Born, 1997). However, postsleep recall rates in the declarative and procedural tasks were almost identical for both the RS-86 and placebo condition (Tables 2 and 3). Furthermore, RS-86-induced alterations in sleep parameters did not correlate with individual changes in pre–post sleep recall rates. Before concluding that M_1 mAChR subtype functioning is not critically implicated in pre–post sleep memory consolidation, a number of potentially confounding factors need to be considered.

The probability that we failed to detect an actually relevant effect of exogenous M_1 mAChR stimulation on postsleep recall rates (false-negative finding) seems low. Both the available pharmacokinetic data (see Methods section) and the alterations in sleep parameters observed in the present study indicate that efficacious RS-86 brain levels were reached during the critical sleep period. Recall performances under RS-86 and placebo conditions were strikingly similar in an intraindividual crossover design comprising 20 healthy participants. Hence, there was no trend suggestive that statistically significant comparisons would emerge with a greater subject number (power problem).

Effects of RS-86 on sleep and memory regulation may be more prominent with alterations in cholinergic neurotransmission, such as those described in depression

or aging (Perlis et al., 2002; Schredl, Weber, Leins, & Heuser, 2001). That is, in healthy subjects, endogenous M_1 mAChR stimulation may be sufficient to support optimal memory consolidation during REM sleep. This possibility may be examined by testing the effects of selective M_1 mAChR antagonism on memory consolidation during sleep. It also may be that an immediate elevation of cholinergic tone posttraining may influence memory processes more effectively than the relatively delayed receptor stimulation used in the present protocol. Studies demonstrating that cholinergic and other memory-modulating drugs are most effective immediately after training, and lose efficacy with time after training, are consistent with this possibility (McIntyre, Power, Roozendaal, & McGaugh, 2003; Izquierdo, 1989).

As a wake control condition was not included in the design of the present study, we did not demonstrate that we observed distinctly sleep-dependent memory consolidation processes. The aim of the present study was to examine possible underpinnings of pre–post sleep memory performance improvement at the level of neurotransmission. The main finding of the present study, the dissociation between sleep and memory parameters after M_1 mAChR stimulation, is, to a large extent, independent from potential wake control findings. However, future sleep–memory research should ultimately include wake control conditions of different time periods at different times of the day to assess the actual contribution of sleep, as well as homeostatic and circadian factors to the consolidation of memories.

We did not observe an impairment of declarative memory consolidation like that reported by Gais and Born (2004) when they administered the AChE inhibitor physostigmine. Therefore, it appears that selective M_1 mAChR stimulation is sufficient to disrupt SWS, but not to concomitantly affect declarative memory consolidation. In the present study, the SWS (%SPT) in the first part of the night was predominantly attenuated. This effect may be related to the pharmacokinetic properties of RS-86 (higher plasma concentration in the first half of the night), intrinsic SWS homeostasis (lower SWS propensity in the second half of the night), or a combination of both. It is worth noting that a critical role of SWS in the first part of the night for the consolidation of declarative memories has been suggested (Plihal & Born, 1997). The present dissociation between disrupted SWS and memory performance, however, is consistent with the findings of Gais and Born in that although they found both SWS and memory consolidation effects in the physostigmine-treated condition, the two effects were found not to be correlated in a within-subject correlational analysis. This dissociation suggests that physostigmine-induced disruption of declarative memory consolidation during sleep is not mediated exclusively, or perhaps even primarily, by M_1 mAChRs. Thus, if the model of cholinergic regulation of information flow between the hippocampus and the neocortex during memory consolidation is accurate (Hasselmo, 1999; Buzsaki, 1996, 1998), then these processes would appear to depend on the contribution, and perhaps the cooperation, of other mAChR subtypes, and possibly nicotinic AChRs. Observations of changes in the expression of multiple mAChR subtypes with aging (Vaucher et al., 2002) and with dementia (Levey, 1996), as well as demonstrations of cooperative effects of mAChR subtypes on behavioral memory (Power et al., 2003), physiological excitation of the hippocampus (Liu, Kumar, & Alreja, 1998), and induction of gene expression (Albrecht et al., 2000) are consistent with this view.

With regard to REM sleep regulation and procedural memory processing, it is important to see that RS-86 did selectively shorten *REM sleep latency* without affecting *REM sleep duration* or the *NREMs* (Table 1). Based on these data and previous studies, we recently proposed that the initiation of REM sleep is mediated, at least to a significant extent, by M_1 mAChRs, whereas the maintenance of REM sleep and the NREMs are mediated by non-M_1 mAChRs, such as M_2 or other mAChRs (Nissen et al., 2006). Thus, it may be that the previously reported association between REM sleep and procedural learning (Plihal & Born, 1997) is related to REM episode duration or eye movement related processes rather than to the timing of REM sleep onset.

Animal studies have strongly implicated M_1 receptors in the hippocampus (Ferreira et al., 2003), amygdala (Power, et al., 2003), striatum, and cortex (Lazareno, Popham, & Birdsall, 2003) in memory. Memory consolidation, in particular, has been shown to be enhanced by M_1 mAChR agonism in rats (Ferreira et al., 2003; Jerusalinsky, Cervenansky, Walz, Bianchin, & Izquierdo, 1993), and memory performance in aged rats correlated with M_1 receptor–G protein coupling in the hippocampus and the neocortex (Rossi, Mash, & de Toledo-Morrel, 2005). In turn, attenuated M_1 mAChR functioning has been linked to disrupted memory processing. Administration of selective M_1 mAChR antagonists impaired the consolidation of long-term memory in rats (Moreira, Ferreira, Fornari, Figueredo, & Oliveira, 2005; Roldan, Bolanos-Badillo, Gonzales-Sanchez, Quirarte, & Prado-Alcala, 1997). And M_1 −/− knockout mice demonstrated selective long-term memory deficits (Anagnostaras et al., 2003). Moreover, spatial memory deficits evoked by cholinergic basal forebrain lesions in rats were attenuated by treatment with a partial (sabcomeline) or a full (RS-86) M_1 mAChR agonist (Hodges, Peters, Gray, & Hunter, 1999). Studies examining interactions between neurotransmitter systems have shown that muscarinic cholinergic activation is critical for modulation of memory consolidation by adrenergic (Power, Thal, & McGaugh, 2002; Introini-Collison, Dalmaz, & McGaugh, 1996), glucocorticoid (Power, Roozendaal, & McGaugh, 2000), and dopaminergic (Lalumiere, Nguyen, & McGaugh, 2004) treatments; and modulation of memory consolidation

appears to require activation of both M_1 and M_2 receptors in the basolateral amygdala (Power et al., 2003).

Despite these preclinical findings suggesting that M_1 mAChR activation may improve memory performance, and despite high densities of M_1 mAChRs in brain regions critical for memory functioning in rodents (Cortes & Palacios, 1986), primates (Mash, White, & Mesulam, 1988), and humans (Cortes et al., 1986), trials in humans investigating the impact of M_1 mAChR stimulation on memory functioning have consistently failed to detect clinically significant effects. Administration of 0.5 to 10 mg of the M_1 mAChR agonist RS-86 in clinical trials of up to 8 weeks in duration did not reveal any improvements in cognitive performance, as assessed by Mini Mental Status, word and picture recognition tasks, and word-list learning (Azonca, Roth, Spiegel, Neff, & Laplanche, 1984; Wettstein & Spiegel, 1984). The findings have thus far consistently been negative in both healthy volunteers (Spiegel, 1985) and patients with conditions associated with a decline in cholinergic neurotransmission, such as in Alzheimer's dementia (e.g., Mouradian, Mohr, Williams, & Chase, 1988; Spiegel, 1985; Wettstein & Spiegel, 1984) or progressive supranuclear palsy (Foster, Aldrich, Bluemlein, White, & Berent, 1989). Moreover, administration of a wide dose range (0.3 to 48 mg) of the M_1 mAChR agonist talsaclidine did not affect memory performance in healthy controls or patients with Alzheimer's dementia (Wienrich et al., 2001). Note that these studies investigated primarily long-term clinical effects and did not further differentiate between different stages of memory encoding or consolidation. The present preponderance of negative findings after M_1 mAChR stimulation suggests that it is unlikely that additional dose groups would have yielded effects on memory performance in the present study. Direct evidence, however, is lacking because the present single-dose study is the first to test the effects of M_1 mAChR agonism on pre–post sleep memory consolidation.

In summary, the present study did not provide support for the hypothesis that selectively enhancing M_1 mAChR functioning during sleep in humans may enhance consolidation of procedural memory while impairing consolidation of declarative memory. In the context of previous negative studies on cognitive performance during waking in humans, it seems worthwhile to further explore the effects of non-M_1 AChR subtypes, as well as cooperative interactions among muscarinic receptor subtypes and among neurotransmitter systems on sleep and wake associated memory consolidation.

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